Supplementary Materials for:

Cytotoxicity of paclitaxel in breast cancer is due to chromosome missegregation on multipolar spindles

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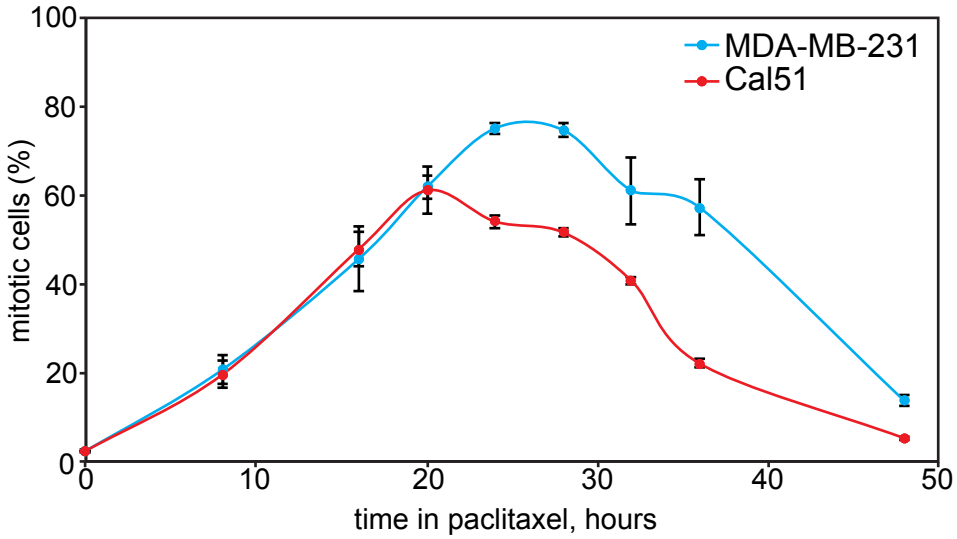

Figure S1. Mitotic index is >15-fold elevated between 16 and 32 hours after paclitaxel administration in breast cancer cells in culture. In response to increasing time in 10 μM paclitaxel, mitotic index in both MDA-MB-231 (blue) and Cal51 (red) cells peaks and then declines. n ≥ 1500 cells from 3 separate experiments.

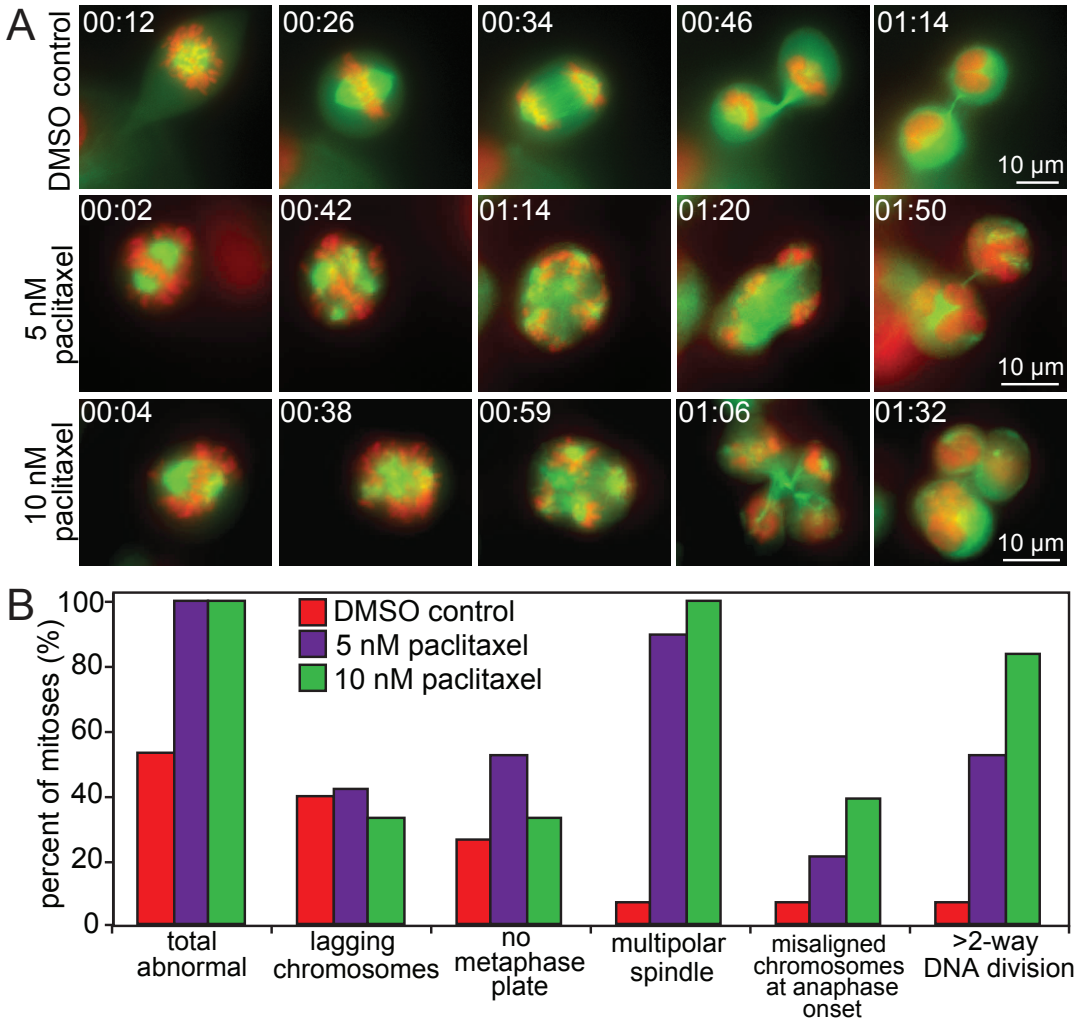

Figure S2. Clinically relevant concentrations of paclitaxel cause abnormal mitoses in MDA-MB-231 cells. (A) MDA-MB-231 cells expressing H2B-RFP (red) and GFP-tubulin (green) were filmed at 60× with 2-minute intervals during mitosis in DMSO (top row), 5 nM (center row), and 10 nM (bottom row) paclitaxel. Shown are still frames from Videos 4-6. Time is shown in hours:minutes. Cells are able to divide in drug although mitotic spindles accumulate additional spindle poles before anaphase onset. (B) Quantitation of mitotic defects in MDA-MB-231 cells. The increase in defects is mainly due to multipolar spindles and >2–way DNA divisions. $n \geq 20$ cells per condition.

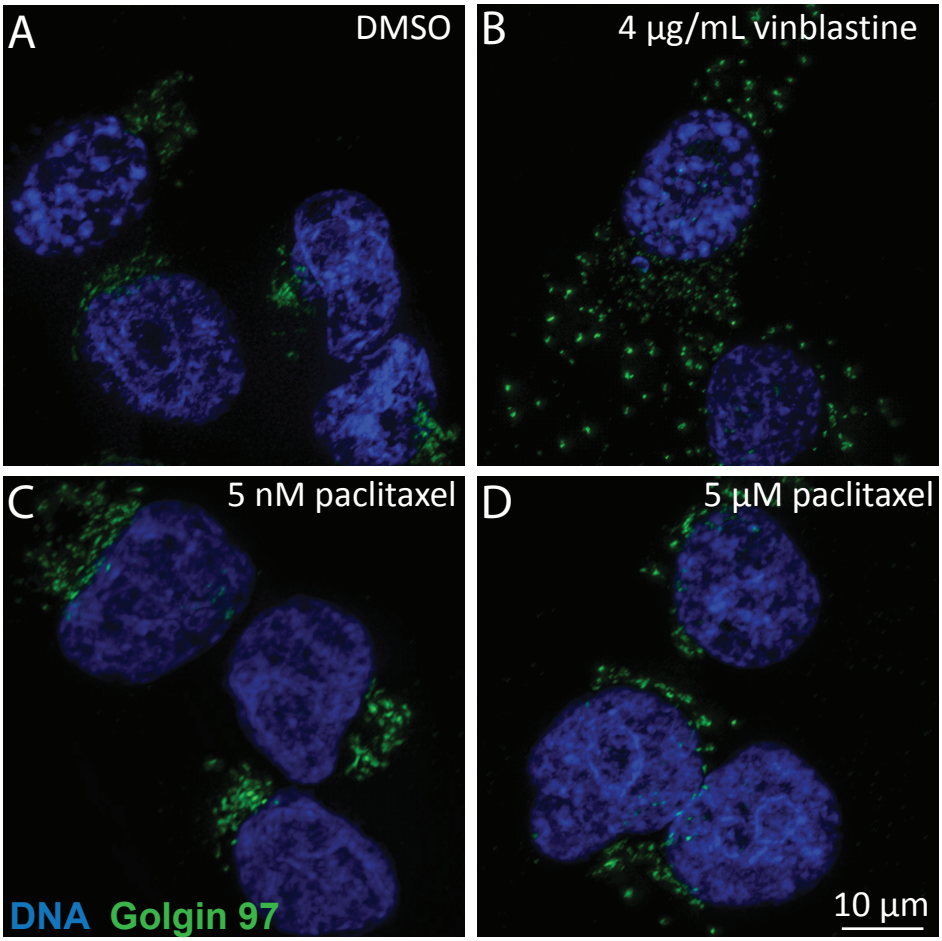

Figure S3. Clinically relevant doses of paclitaxel do not disrupt Golgi structure. Representative images of MDA-MB-231 cells stained with antibody against Golgin-97 (green) and DAPI (blue). (A) Normal Golgi distribution in cells treated with vehicle alone (DMSO). (B) Vinblastine treatment disperses the Golgi due to microtubule depolymerization. (C) Golgi distribution is unaffected by treatment with a clinically relevant dose of paclitaxel (5 nM). (D) 5 µM paclitaxel has subtle effects on Golgi distribution as compared to vinblastine.

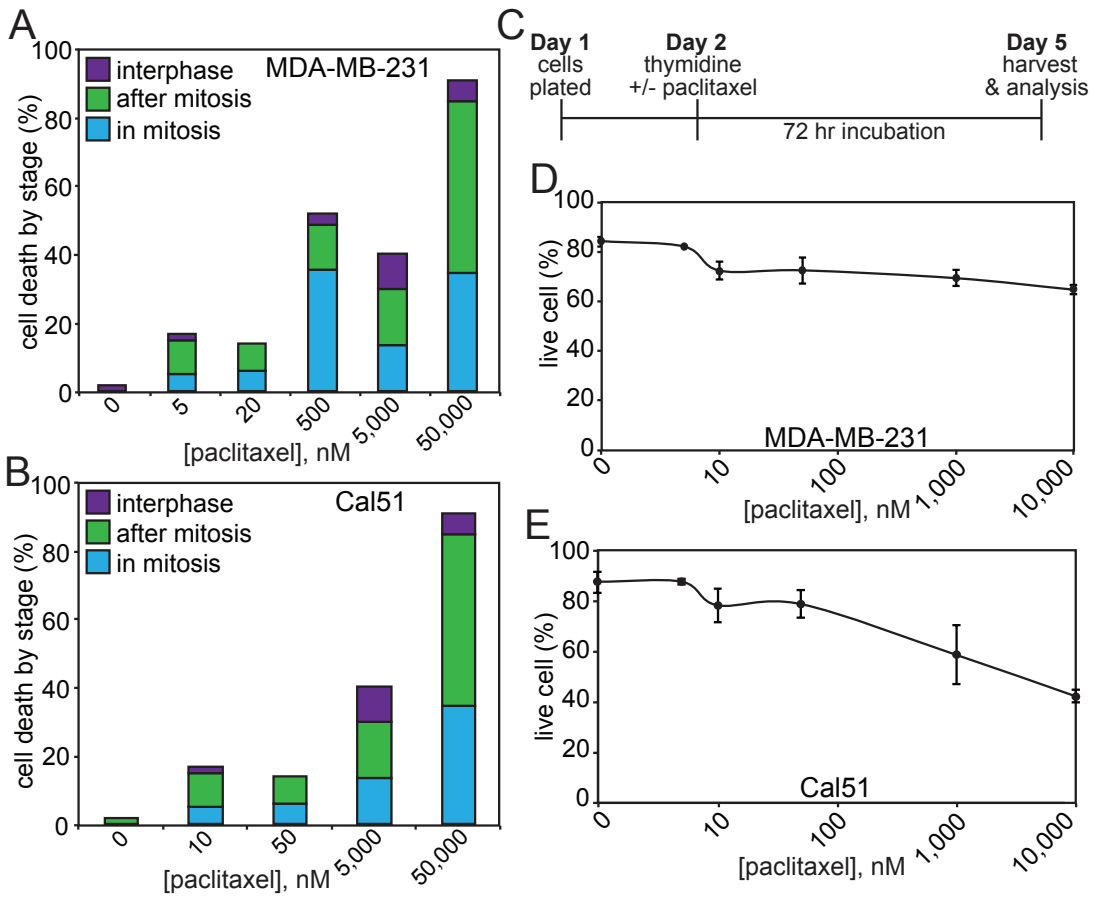

Figure S4. Clinically relevant doses of paclitaxel do not cause substantial cell death from interphase. (A and B) Cells were observed by phase-contrast timelapse microscopy for 65 hours to ascertain the frequency of cell death and the cell cycle stage at time of death. In both MDA-MB-231 (A) and Cal51 (B) cells, death in paclitaxel occurs primarily during or following exit from mitosis, not in cells that remained in interphase during the duration of paclitaxel treatment. (C) Schematic of experiment to test whether paclitaxel can kill interphase cells without passage through mitosis. (D-E) Clinically relevant paclitaxel concentrations do not cause substantial cell death in MDA-MB-231 (D) or Cal51 (E) cells arrested in S phase. High doses of paclitaxel cause cell death in interphase Cal51, but not MDA-MB-231, cells.

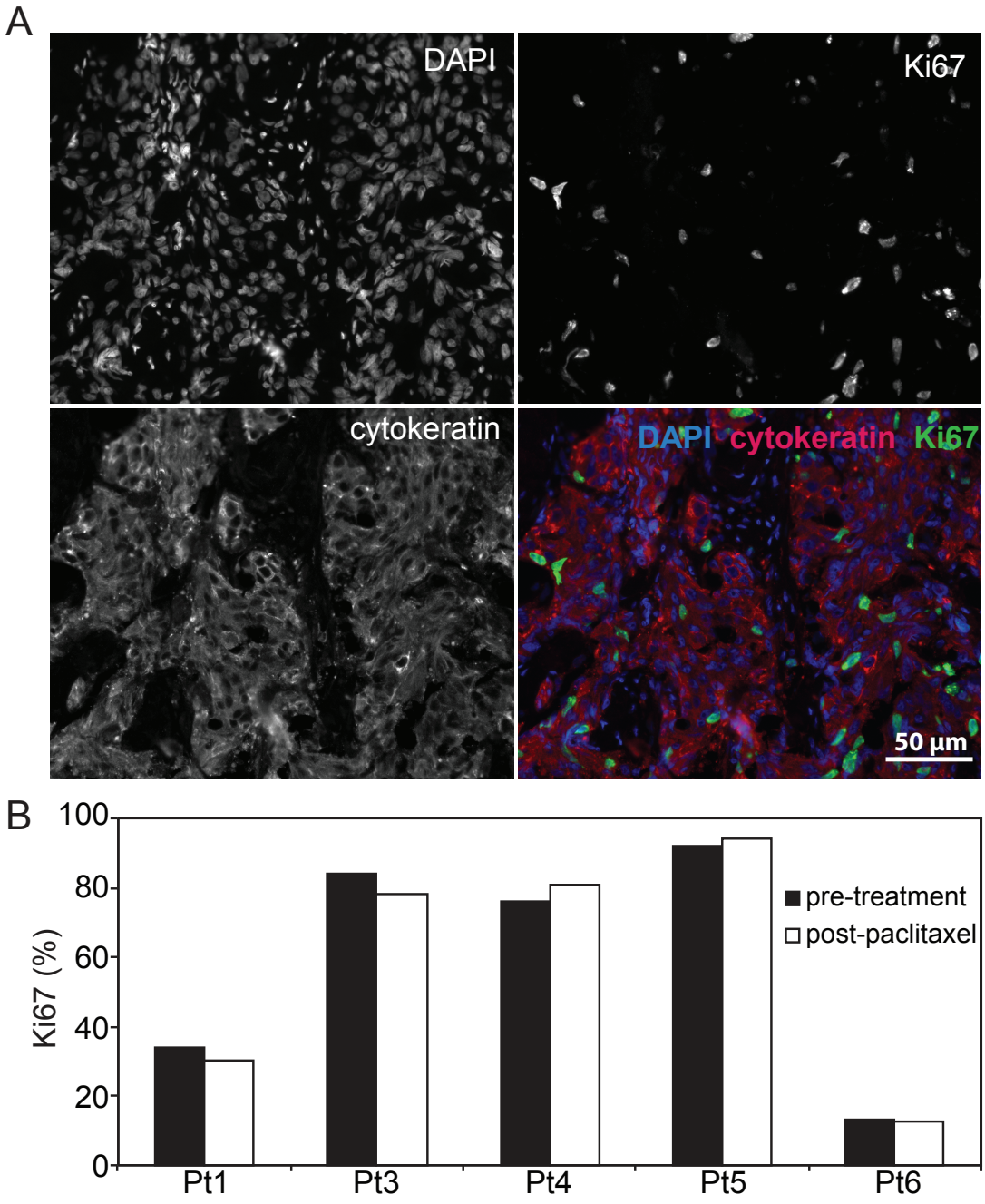

Figure S5. The proliferative index in patient tumors is unchanged by paclitaxel treatment. (A) Immunofluorescence images of a 5 µm section from a diagnostic biopsy of a patient tumor. Ki67 staining, used as a marker to distinguish G1, S and G2 cells from quiescent cells, green. Cytokeratin, used to identify epithelial cells, red. Nuclei are stained with DAPI, blue. (B) Percentage of epithelial cells positive for Ki67 before or after 20 hours of paclitaxel treatment. Most tumors, including those that responded to paclitaxel, showed a proportion of Ki67 positive cells substantial enough for paclitaxel to exert its cytotoxic effects on mitotic, rather than interphase, cells. n > 500 cells per condition.

Table S1. Paclitaxel measurements in patients by tumor volume.

patient #	plasma [paclitaxel], μM	tumor [paclitaxel], μM	degree of concentration
1	0.08	2.5	31×
2*	-	-	-
3	0.14	12.0	86×
4	0.11	7.8	71×
5	0.28	1.3	5×
6	0.15	2.2	15×

*skin biopsy of superficial tumor that yielded minimal tumor tissue
- = not tested.

Table S2. Statistical p values from figures 3 and 4.

Fig 3A					
MDA-MB-231					
	5 nM	**10 nM**	**20 nM**	**50 nM**	**100 nM**
24 hr	n/s	n/s	1.17E-02	1.06E-03	4.15E-03
72 hr	2.66E-02	2.66E-02	2.70E-03	2.70E-03	1.67E-02
120 hr	2.68E-03	1.73E-03	1.75E-03	1.73E-03	8.15E-03
Cal51					
	5 nM	**10 nM**	**20 nM**	**50 nM**	**100 nM**
24 hr	n/s	n/s	1.33E-02	9.38E-03	1.36E-02
72 hr	4.72E-02	8.74E-03	4.00E-03	2.66E-03	1.64E-02
120 hr	2.50E-02	3.95E-03	3.95E-03	3.95E-03	2.01E-02
Fig 3B					
MDA-MB-231					
	5 nM	**10 nM**	**20 nM**	**50 nM**	**100 nM**
24 hr	4.38E-02	2.63E-03	n/s	2.00E-06	9.17E-05
72 hr	3.60E-02	5.33E-06	5.39E-11	8.30E-20	9.86E-13
120 hr	4.41E-06	5.41E-18	3.44E-27	3.68E-37	2.65E-25
Cal51					
	5 nM	**10 nM**	**20 nM**	**50 nM**	**100 nM**
24 hr	n/s	2.86E-02	4.20E-03	1.38E-04	1.26E-04
72 hr	n/s	9.50E-03	7.17E-07	6.86E-12	8.79E-22
120 hr	1.62E-02	1.83E-04	7.86E-06	1.39E-09	4.22E-16
Fig 3C					
MDA-MB-231					
	5 nM	**10 nM**	**20 nM**	**50 nM**	**100 nM**
	4.95E-02	4.63E-02	4.63E-02	4.63E-02	4.63E-02
Cal51					
	5 nM	**10 nM**	**20 nM**	**50 nM**	**100 nM**
	4.95E-02	4.95E-02	4.95E-02	4.95E-02	4.63E-02
Fig 4B					
	5 nM	**10 nM**	**50 nM**		
	4.84E-03	5.79E-03	4.50E-05		

*all values are 2-sided
n/s = not statistically significant

Supplementary Videos

Video S1. Normal bipolar division in a control Cal51 breast cancer cell. In the chromosomally stable, triple negative Cal51 breast cancer cell treated with vehicle alone (DMSO), a bipolar division results in the formation of 2 daughter cells.

Video S2. Abnormal division in a Cal51 cell treated with 10 nM paclitaxel. A pseudo-bipolar spindle segregates DNA to four distinct poles. Two daughter cells are produced.

Video S3. Abnormal division in a Cal51 cell treated with 10 nM paclitaxel. After a brief delay on a highly multipolar spindle, DNA is segregated in multiple directions. Cytokinesis fails altogether and a single cell is ultimately generated.

Video S4. Normal division in an MDA-MB-231 cell. A triple negative, chromosomally unstable MDA-MB-231 breast cancer cell proceeding through mitosis after treatment with vehicle alone (DMSO). The cells enter anaphase with a bipolar mitotic spindle producing a 2-way DNA division and 2 daughter cells.

Video S5. Abnormal division in an MDA-MB-231 cell treated with 5 nM paclitaxel. After the cell initially forms a bipolar spindle, additional spindle poles arise. At anaphase onset, the DNA divides in four directions and the resulting DNA masses coalesce to form two daughter cells.

Video S6. Abnormal division in an MDA-MB-231 cell treated with 10 nM paclitaxel. The initially bipolar spindle becomes multipolar over time. A 4-way DNA division during anaphase ultimately produces three daughter cells.